Form PTO-1390 (Rev. 12-29-99) US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NO. C 2178 PCT/US
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 10/009316
INTERNATIONAL APPLICATION NO. PCT/EP00/03764	INTERNATIONAL FILING DATE April 26, 2000
	PRIORITY DATES CLAIMED May 5, 1999 & May 28, 1999

TITLE OF INVENTION

METHOD FOR THE SELECTIVE ESTERIFICATION OF POLYOLES

APPLICANT(S) FOR DO/EO/US

Uwe Bornscheuer, Rolf Schmid, Christoph Syldatk, Youchun Yan, Ralf Otto

Applicant herewith submits to the United States Designated/Elected Office (EO/DO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. □ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. □ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).

4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. □ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. □ is not required, as the application was filed in the United States Receiving Office (RO/US).

6. ■ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. □ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. □ have been transmitted by the International Bureau.
   c. □ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.

8. □ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ■ An oath or declaration of the Inventor(s) (35 U.S.C. 371(c)(4)). **(UNEXECUTED)**

10. □ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. □ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. □ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ■ A FIRST preliminary amendment
    □ A SECOND or SUBSEQUENT preliminary amendment.

14. □ A substitute specification.

15. □ A change of power of attorney and/or address letter.

16. □ Other items or information:

"Express Mail Post Office to Addressee" service Mailing Label Number EL541613973US .

JC07 Rec'd PCT/PTO 05 NOV 2001

U.S. Application No. (if known, see 37 CFR 1.5) 10/009316	INTERNATIONAL APPLICATION NO. PCT/EP00/03764	ATTORNEY'S DOCKET NUMBER C 2178 PCT/US

17.■ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1,040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date 37 (CFR 1.492(e)).	$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	13 - 20 =	0	0 X $18.00	$ 0	
Independent Claims	2 - 3 =	0	0 X $84.00	$ 0	
Multiple dependent claims (s)(if applicable)		0	+ $280.00	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 890	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0	
SUBTOTAL =				$ 890	
Processing fee of **$130.00** for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 890	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 890	
				Amount to be: refunded:	$----------
				charged:	$890.00

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ■ Please charge my Deposit Account No. 50-1177 in the amount of $890.00 to cover the above fees. A triplicate copy of this sheet is enclosed. Order No. 01-0698 .

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1177 . A triplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Cognis Corporation, Law Dept.
2500 Renaissance Blvd., Suite 200
Gulph Mills, PA 19406

SIGNATURE:

John E. Drach
NAME ATTORNEY FOR APPLICANT

32,891
REGISTRATION NUMBER

"Express Mail" mailing label number EL541613973US .

PATENT
Docket No. C 2178 PCT/US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE: PCT/EP00/03764
International Filing Date: April 26, 2000
Priority Dates Claimed: May 5, 1999 & May 28, 1999
Applicant: Bornscheuer, et al.
Title: METHODS FOR THE SELECTIVE ESTERIFICATION OF POLYOLES
Applicants' Reference: C 2178 PCT/US

PRELIMINARY AMENDMENT

Commissioner for Patents
Box PCT
Washington, DC 20231

ATTN: DO/EO/US

Sir:

Before examination, in the national stage for the United States, of the above-captioned application under the Patent Convention Treaty, please amend as follows the translation supplied herewith of the application:

In the Specification:

Please delete all text above line 3, of page 1, and replace the deleted matter with the following new section headings and new paragraph:

--TITLE OF THE INVENTION

Method for the Selective Esterification of Polyols

BACKGROUND OF THE INVENTION

This invention relates to a process for the enzyme-catalyzed production of carboxylic acid esters of polyhydric alcohols.--

Please delete the paragraph beginning on line 21, page 4 and ending on line 25, page 4 and replace the deleted matter with the following new paragraph:

--SUMMARY OF THE INVENTION

Preliminary Amendment of U.S. National Stage for International Application PCT/EP00/03764 filed on April 26, 2000

The present invention relates to a process for the production of polyols, more particularly sugars or sugar derivatives, esterified with carboxylic acids at the primary OH group, characterized in that the polyol is reacted with a carboxylic acid ester in the presence of an organic solvent and a hydrolase, preferably a lipase or esterase, as catalyst.--

Please delete the paragraph beginning on line 26, page 4 and ending on line 8, page 5 and replace the deleted matter with the following new paragraph:

--DETAILED DESCRIPTION OF THE INVENTION

A feature of the polyols in the context of the present invention is that they have a primary alcohol function and, in addition, at least one other secondary or tertiary alcohol function. More particularly, they are sugars or sugar derivatives. Examples include threose, erythrose, arabinose, lyxose, ribose, xylose, allose, altrose, galactose, glucose, gulose, idose, mannose, talose and fructose and di-, oligo- and optionally polymers composed of them. Useful sugar derivatives include, for example, the oxidized derivatives of the compounds mentioned, such as the aldonic acids and ascorbic acid. The naturally occurring isomers of the sugars, mostly the D-forms, are preferred. It is crucial to the invention that, besides the primary alcohol group necessary for the esterification reaction, these compounds are used with at least one free, i.e. unprotected, secondary or tertiary alcohol function.--

On a separate, new page 14, following page 13, please add the following new section heading and paragraph containing an Abstract of the Disclosure:

--ABSTRACT OF THE DISCLSOURE

Polyols that are derivatives of sugars or sugar derivatives are produced by selectively esterifying the primary OH group of the sugar or sugar derivative with carboxylic acid ester in the presence of an organic solvent and a hydrolase.--

In the claims:

Please cancel claims 1-13.

Preliminary Amendment of U.S. National Stage for International Application PCT/EP00/03764 filed on April 26, 2000

Please add the following new claims 14-26.

14. (New) A process for the production of sugar derivatives selectively esterified with carboxylic acids at the primary OH group comprising, reacting a sugar derivative selected from the group consisting of an aldonic acid and ascorbic acid with a carboxylic acid ester in the presence of an organic solvent and a hydrolase.

15. (New) The process of claim 14 wherein the hydrolase is a lipase or esterase.

16. (New) The process of claim 1 wherein the hydrolase is an enzyme obtainable from Candida antarctica, Humicola lanuginosa, Rhizopus spec., Chromobacterium viscosum, Aspergillus niger, Candida rugosa, Penicillium camembertii, Rhizomucor miehei, Burkholderia spec. or Pseudomonas spec.

17. (New) The process of claim 1 wherein the hydrolase is deposited on a solid support material.

18. (New) The process of claim 1 wherein the carboxylic acid is a compound of the formula R-COOH wherein R is an alkyl or alkenyl group having from about 6 to about 32 carbon atoms; a hydroxysubstituted alkyl or alkenyl group having from about 6 to about 32 carbon atoms; AR-$(CH_2)_n$ wherein AR is a phenyl or naphthyl group, a hydroxysubstituted phenyl or naphthyl group; and n is a number of 0 to 4.

19. (New) The process of claim 1 wherein the carboxylic acid ester is a lower alkyl ester selected from the group consisting of the methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl and tert. butyl ester.

20. (New) The process of claim 1 wherein the mole ratio of the carboxylic acid ester to the sugar is from about 0.8 to about 1.2:1.

21. (New) The process of claim 1 wherein the weight of the organic solvent is 0.1

to 25 times greater than the weight of sugar derivative.

22. (New) The process of claim 21 wherein the weight of the organic solvent is 0.5 to 18 times greater than the weight of sugar derivative.

23. (New) The process of claim 1 wherein the organic solvent is selected from dioxane, acetonitrile, acetone, γ-butyrolactone, tetrahydrofuran, tert. butanol, tert. amyl alcohol and 3-methyl-3-pentanol and mixtures thereof.

24. (New) The process of claim 1 wherein the process is carried out at temperature from room temperature to about 80°C.

25. (New) The process of claim 1 wherein the process is carried out at temperature from room temperature to about 60°C.

26. (New) A process for the production of sugar derivatives selectively esterified with carboxylic acids at the primary OH group comprising reacting a sugar derivative selected from the group consisting of an aldonic acid and ascorbic acid with a carboxylic acid ester in the presence of an organic solvent and a hydrolase and removing the alcohol formed in the reaction by azeotropic distillation.

Preliminary Amendment of U.S. National Stage for International Application PCT/EP00/03764 filed on April 26, 2000

REMARKS

Claims 14-26 are currently pending in the instant application.

The Specification has been amended to include the preferred section headings pursuant to 37 C.F.R. §1.77. An Abstract of the Disclosure has been added on a separate sheet following the claims. It is submitted that the amendments to the Specification made herein introduce no new matter. Their entry is therefore proper and respectfully requested.

Original claims 1-13 have been canceled and replaced with new claims 14-26 solely for the purpose of improving clarity and grammar, which may suffer in translation, and not for any reason related to the statutory requirements for a patent. New claims 14-26 have not been added in response to any rejection, or in anticipation of any rejection related to the statutory requirements for a patent. Applicants respectfully submit that the scope of new claims 14-26 corresponds to the scope of original claims 1-13 and that new claims 14-26 are no narrower than original claims 1-13. Furthermore, although a moot point in view of their cancellation, Applicants respectfully submit that original claims 1-13 satisfied the requirements of 35 U.S.C. §112, as filed. New claims 14-26 are supported by the specification and no new matter has been introduced. Entry is therefore proper and respectfully requested. Prompt examination of the instant application in view of the amendments made herein is respectfully requested.

Respectfully submitted,

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ABSTRACT OF THE DISCLOSURE

Polyols that are derivatives of sugars or sugar derivatives are produced by selectively esterifying the primary OH group of the sugar or sugar derivative with carboxylic acid ester in the presence of an organic solvent and a hydrolase.

Method for the Selective Esterification of Polyoles

This invention relates to a process for the enzyme-catalyzed production of carboxylic acid esters of polyhydric alcohols.

Chemically produced surfactants are generally made up of alkyl or aryl groups which, in the case of ionic surfactants, contain carboxylate, sulfonate, phosphate or ammonium groups and, in the case of nonionic compounds, alcohol or polyether groups or sugar units to enhance solubility in water. The advantage of such surfactants is their relatively simple and inexpensive production which has been optimized over many decades on an industrial scale. One of their disadvantages is the relatively limited range of variation of the functional groups in the lipophilic part of the molecule. Another common disadvantage is that a large part is still dependent on petroleum as the raw material base. Accordingly, corresponding surfactants are only used to a limited extent in foods and in pharmaceutical products. In detergents/cleaners and in cosmetics, at least half the surfactants used today are based on natural oils and fats. In contrast to the so-called chemical surfactants, so-called biosurfactants show a wide diversity of structure not only in the hydrophilic but also in the lipophilic part of the molecule (**S. Lang and F. Wagner in: *Biosurfactants and Biotechnology*, Ed.: N. Kosaric, W.L. Cairns and N.C.C. Gray, Marcel Dekker, New York, 1987, 25, 21-46**). They are mostly microbial secondary metabolites that are preferentially formed by product strains when grown on lipophilic substrates, such as n-alkanes or triglycerides. Besides favorable environmental compatibility, these compounds often exhibit interesting biological effects such as, for example, membrane activity or antibiotic activity which make them appear increasingly interesting for industrial use in the pharmaceutical, cosmetic and food sectors. Hitherto, vegetable or animal biosurfactants produced by

elaborate methods have been almost exclusively used in those sectors (**V. Klekner and N. Kosaric in: *Biosurfactants: Production-Properties-Applications*, Ed.: N. Kosaric, Marcel Dekker, New York, 1993, 48, 373-390**). There is a demand there for more simple methods of production which provide such substances in high yields and purities.

The production of sugar esters of aliphatic carboxylic acids by standard methods of chemical synthesis is known (**J.C. Colbert, *Sugar Esters - Preparation and Application*, Noyes Data Corporation, New Jersey 1974**). The chemical preparation of esters of unprotected sugars, i.e. compounds containing several free alcohol functions, and carboxylic acids generally leads to unspecific mixtures of mono- and polyacylated sugars so that protective groups have to be introduced and removed if a certain product is to be selectively synthesized. The use of activated carboxylic acid derivatives, such as acid chlorides or anhydrides, inevitably results in the formation of by-products and, in many cases, unwanted secondary products which pollute the environment, complicate working up and reduce the yields of desired product. The production of sugar esters of aromatic carboxylic acids by such standard methods of chemical synthesis is also known (**A.F. Artamonov, L.F. Burkovskaya and G.V. Nikonov, *Khim. Prir. Soedin* 1994, 4, 561-562**) and is similarly attended by the above-mentioned disadvantages.

Another method described in the literature for producing esters of sugars or glycosides and aromatic carboxylic acids are biotransformations with plant cell cultures (**M. Ushiyama, S. Kumagai and T. Furuya, *Phytochemistry* 1989, 28, 3335-3339**). However, these authors merely describe analytical yields because the sugar esters are presumably converted rapidly into other components by degradation and further reactions so that this approach is of no economic value.

The most widely described method of obtaining aromatic esters of sugars or glycosides and aromatic carboxylic acids is isolation from

naturally occurring sources, more especially plants (**P.C. Lyons, K.V. Woods and R.L. Nicholson, *Phytochemistry* 1990 29, 97-101; H. Shimomura, Y. Sashida, M. Oohara and H. Teuma, *Phytochemistry* 1988, 27, 644-646; Y. Kashiwada, G.I. Nonaka, I. Hishioka and T. Yamagashi, *Phytochemistry* 1988, 27, 1473-1477; M. Nicoletti, C. Galeffi, I. Messana, G.B. Marini-Bettolo, J.A. Gabarino and V. Gambaro, *Phytochemistry* 1988, 27, 639-641; Y. Kashiwada, G.I. Nonaka and I. Hishioka, *Chem. Pharm. Bull.* 1984, 32, 3461-3470**). Low yields and the use of - in some cases - highly toxic solvents complicate access to the target compounds. In addition, where this method is adopted, production is limited to the naturally occurring representatives so that structurally even slightly modified esters cannot be obtained in this way.

In nature, the formation of such esters is the last step of a biosynthesis route which is catalyzed by various enzymes from the group of acyltransferases. These enzymes show relatively high flexibility in regard to the acyl group, but very strict selectivity for the alcohol substrate to be esterified. A considerable disadvantage is that they need stoichiometric quantities of the corresponding acyl coenzyme A which makes them unsuitable in practice for the *in vitro* synthesis. Nevertheless, the enzymatic coupling of aliphatic fatty acids onto simple sugars with the aid of such enzymes has been described. The problem of the poor solubility and miscibility of sugars and fatty acids was overcome here by various methods: i) using polar solvents, such as pyridine or dimethyl formamide (**J. Chopineau, F.D. McCafferty, M. Therisod and A.M. Klibanov, *Biotechnol. Bioeng.* 1988, 31, 208-214**), ii) introducing protective groups, such as isopropylidene acetals or phenyl boric acid esters, in order to increase the solubility of the sugar component in organic solvents (**K. Adelhorst, F. Björkling, S.E. Godtfredsen and O. Kirk, *Synthesis* 1990, 112-115; C. Scheckermann, A. Schlotterbeck, M. Schmidt, W. Wray**

and S. Lang, *Enzyme Microb. Technol.* 1995 17, 157-162), iii) using activated acyl donors to increase the reaction rate (**M. Therisod and A.M. Klibanov. *J. Am. Chem. Soc.* 1986, 108, 5638-5640**), iv) reaction in a substantially solid system in the presence of small quantities of an added organic solvent (**L. Cao, A. Fischer, U.T. Bornscheuer and R.D. Schmid, *Biocatal. Biotransform.* 1997, 14, 269-283**).

Disadvantages of methods i) and ii) are the inactivation of the enzyme by the solvent, the need for additional synthesis steps to introduce and remove protective groups, poor yields and the use of solvents which seriously restrict the use of the reaction products in certain fields of application, for example the pharmaceutical and food sectors. A potential disadvantage of method iv) in particular was found to be that the working up of the reaction products from a substantially solid reaction mixture is often not possible without losses and that, in addition, considerable difficulties are involved in carrying out the reaction continuously where this procedure is adopted.

It has now surprisingly been found that the use of a hydrolase and small quantities of an organic solvent enables corresponding esters to be selectively obtained from polyols, such as sugars or sugar derivatives, and nonactivated carboxylic acid derivatives.

The present invention relates to a process for the production of polyols, more particularly sugars or sugar derivatives, esterified with carboxylic acids at the primary OH group, characterized in that the polyol is reacted with a carboxylic acid ester in the presence of an organic solvent and a hydrolase, preferably a lipase or esterase, as catalyst.

A feature of the polyols in the context of the present invention is that they have a primary alcohol function and, in addition, at least one other secondary or tertiary alcohol function. More particularly, they are sugars or sugar derivatives. Examples include threose, erythrose, arabinose, lyxose, ribose, xylose, allose, altrose, galactose, glucose, gulose, idose, mannose,

talose and fructose and di-, oligo- and optionally polymers composed of them. Useful sugar derivatives include, for example, the oxidized derivatives of the compounds mentioned, such as the aldonic acids and ascorbic acid. The naturally occurring isomers of the sugars, mostly the D-forms, are preferred. It is crucial to the invention that, besides the primary alcohol group necessary for the esterification reaction, these compounds are used with at least one free, i.e. unprotected, secondary or tertiary alcohol function.

The carboxylic acids to be esterified with the polyols mentioned preferably correspond to the general formula R-COOH, where R is an optionally hydroxysubstituted alkyl or alkenyl group containing 6 to 32 carbon atoms or AR-$(CH_2)_n$ and AR is an optionally alkyl- or hydroxysubstituted phenyl or naphthyl group and n is a number of 0 to 4. Preferred representatives include caproic acid, oenanthic acid, caprylic acid, pelargonic acid, capric acid, lauric acid, lauroleic acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic acid, petroselic acid, petroselaidic acid, oleic acid, elaidic acid, ricinoleic acid, linoleic acid, linolaidic acid, linolenic acid, elaeostearic acid, arachic acid, gadoleic acid, arachidonic acid, behenic acid, erucic acid, brassidic acid, clupanodonic acid, lignoceric acid, cerotic acid, melissic acid, phenylacetic acid, phenylbutyric acid, phenylvaleric acid and meta-hydroxyphenylacetic acid. They are used in the form of nonactivated derivatives, more particularly in the form of their alkyl, alkylphenyl or alkenyl esters, lower esters, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert.butyl or vinyl esters being particularly preferred.

The molar ratio between the nonactivated carboxylic acid derivative and the polyol used in the process according to the invention preferably deviates very little from 1:1 and, in one particular embodiment, is in the range from 0.8 to 1.2:1 because the highest yields of desired product and the lowest amounts of secondary products are obtained in that range.

According to the invention, organic solvent is normally used in quantities of about 0.1 to 25 times and more particularly 0.5 to 18 times the quantity by weight of polyol to be esterified. In a preferred embodiment of the process according to the invention, the educts to be reacted with one another are reacted in a first solvent which readily dissolves both educts and, on completion of the reaction, a second solvent in which the product formed is sparingly soluble is added. Suitable organic solvents include, for example, dioxane, acetonitrile, acetone, ethyl methyl ketone, γ-butyrolactone, tetrahydrofuran, tert.butanol, tert.amyl alcohol and 3-methyl-3-pentanol and mixtures thereof, tert.butanol being a particularly preferred first solvent and acetone being a particularly preferred second solvent. In a preferred embodiment of the process according to the invention, an ester, for example a methyl ester, is used as the nonactivated carboxylic acid derivative, releasing an alcohol, for example methanol, after reaction with the polyol. This alcohol is removed from the reaction mixture by azeotropic distillation. In this variant of the process, the solvent, for example acetone, is selected so that it forms an azeotrope with the alcohol to be removed.

Suitable lipases include, for example, the enzymes obtainable from Candida antarctica, Humicola lanuginosa, Rhizopus spec., Chromobacterium viscosum, Aspergillus niger, Candida rugosa, Penicillium camembertii, Rhizomucor miehei, Burkholderia spec. or Pseudomonas spec. They are preferably used in solid form, i.e. immobilized on a support material in known manner.

The process according to the invention is preferably carried out at temperatures in the range from room temperature to 80°C, more particularly at 60°C.

On completion of the reaction, the desired product can be isolated from the reaction mixture by standard methods, for example by extraction with a suitable solvent and optionally further purification, for example by crystallization or chromatography on silica gel.

The process according to the invention allows the chemo- and regioselective synthesis of a broad spectrum of organic compounds which hitherto were difficult to obtain or had never been described before and which are of interest for use in the cosmetic, food, pharmaceutical and environmental sectors.

In the light of the prior art cited above, especially based on experience with chemical reactions, it had been expected that production from unprotected sugars and fatty acid derivatives, such as fatty acid esters, would lead to unspecific mixtures of mono- or polyacylated sugar esters accompanied by the disadvantages mentioned above. In addition, conditions that even allow the reaction of sensitive substrates, such as vitamin C, without destruction by oxidation (a typical problem of chemical methods) were developed by means of the reaction according to the invention.

Moreover, it must be emphasized that, by only slight variation of the reaction conditions, the reaction according to the invention enables a very broad range of different products to be produced in better yields and purities and under milder conditions than is possible by methods known from the prior art.

The products obtainable by the process according to the invention have a surfactant structure, i.e. they consist of a water-soluble hydrophilic molecule part and at least one readily liposoluble hydrophobic molecule part. The size ratio of the molecule parts to one another (hydrophilic/lipophilic balance or HLB value) and the functional groups present therein determine the surfactant properties of the particular compound. The reaction according to the invention allows a very wide range of variation in the linking of different structural elements and hence the simple production of compounds with different HLB values. It is thus possible to produce surface-active emulsifiers both for water-in-oil and for oil-in-water emulsions - a spectrum which is of considerable interest for

applications in the cosmetic, pharmaceutical, food and environmental sectors.

The surface activity of the compounds produced by the process according to the invention is at least comparable with that of aliphatic sugar esters produced by chemical or fermentative methods. Particular emphasis is placed on the improved solubility of the products obtained in accordance with the invention in water. They are suitable for use as emulsifiers, particularly for oil-in-water emulsions, and as surface-active constituents in detergents/cleaners. The surface-active properties can readily be influenced by the choice of suitable acyl donors. In addition, the compounds are readily biodegradable.

Compounds obtainable by the process according to the invention show diverse pharmaceutical activity. Biosurfactants demonstrably show antibiotic effects and membrane activity. In addition, the reaction offers other interesting possibilities because it enables active substances to be given a more hydrophobic or more hydrophilic character. Thus, aromatic carboxylic acids can be made accessible to infusion therapy via glycosylation. On the other hand, hydrophilic substances, such as vitamin C or glycosides, can be esterified with hydrophobic carboxylic acids so that they can be dissolved in creams or anchored in biological membranes.

Glucose esters can be found in therapeutically active plants, such as Prunus spec., Rheum spec. or Thymus spec., which are used for the treatment of bacterial and viral infections, such as colds or headaches, and also disorders of the heart and digestive tract. They play an important part in traditional Chinese medicine. This explains why the glucose esters are isolated by botanical institutes and investigated for their activity (**O.M. Abdallah, M.S. Kamel and M.H. Mohamed, *Phytochemistry* 1994, 37, 1689-1692; . Budzianowski and L. Skrzypezak, *Phytochemistry* 1995, 38, 997-1001; M. Ushiyama, S. Kumagai and T. Furuya, *Phytochemistry* 1989, 28, 3335-3339; Y. Kashiwada, G.I. Nonaka and I.**

Nishioka, *Chem. Pharm. Bull.* 1984, 32, 3461-3470). Important examples of the therapeutic application of the esters obtainable by the process according to the invention are the effect on the arachidonic acid metabolism in leucocytes by caffeoylglucose (**Y. Kimura, H. Okada, S. Nishibe and S. Arichi, *Plant Med.* 1987, 53, 148-153**), the prevention of metastasis formation by galloylglucose (**N. Ata, T. Oku, M. Hattori, M. Fujii, M. Nakajima and I. Saiki, *Oncol. Res.* 1996, 8, 503-511**) and the inhibition of herpes simplex replication after infusion of Verbascum thapsiforme infusions containing aromatic glucose esters (**A. Slagowska, I. Zgorniak-Nowosielska and J. Grzybek, *Pol. J. Pharmacol. Pharm.* 1987, 39, 55-61**). The process according to the invention enables adequate quantities of substance to be provided for pharmacological studies and a broad range of applications.

Examples

Example 1: preparation of 6-O-palmitoyl-β-D-glucopyranose (**B1**)

5 mmol D-glucose and 5 mmol palmitic acid methyl ester (defined here as 1 part by weight) in twice the quantity of tert.butanol based on weight (i.e. corresponding to 2 parts by weight) were heated with stirring (magnetic stirrer, 250 r.p.m.) to ca. 75°C and kept at that temperature throughout the reaction. 0.15 part by weight of immobilized *Candida antarctica* B lipase (SP 435, manufacturer Novo Nordisk) was then added. The progress of the reaction was followed by thin-layer chromatography. After the end of the reaction, 10 parts by weight of warm (ca. 50°C) acetone were added and the mixture was filtered at 50°C. The filtrate was cooled to -10°C and the product **B1** precipitating was isolated by filtration in a yield of 49%. Melting point: 135-136°C.

^{1}H-NMR ([D$_6$]DMSO/TMS): δ (ppm) = 1.03 (t, 3H, H-16´), 1.44 (m, 24H, H-4´ to H-15´), 1.69 (m, 2H, H-3´), 2.45 (t, 2H, H-2´), 3.21 (m, 1H, H-4), 3.31

(m, 1H, H-2), 3.60 (m, 1H, H-3), 3.95 (m, 1H, H-5), 4.18 (dd, 1H, I = 6,23 Hz, I = 11,64 Hz, H-6a), 4.44 (d, 1H, I = 11.46 Hz, H-6b), 4.71 (d, 1H, I = 6.75, OH-3 or OH-2), 4.94 (d, 1H, I = 4.82, OH-4), 5.08 (dd, 1H, I = 4.10, I = 3.97, H-1), 5.22 (d, 1H, I = 5.67, OH-2 or OH-3), 6.53 (d, 1H, I = 4,61, OH-1).

^{13}C-NMR ([D_6]DMSO): δ (ppm) = 13.11 (C-16′, CH_3), 21.27 (C-15′, CH_2), 23.64 (C-3′, CH_2), 27.62 (C-4′, CH_2), 27.89 (C-5′, CH_2), 27.91 (C-6′, CH_2), 28.10 (C-7′, CH_2), 28.19 (C-8′, C-9′, CH_2), 28.23 (C-10′, C-11′, C-12′, C-13′,CH_2), 30.47 (C-14′, CH_2), 32.60 (C-2′, CH_2), 63.04 (C-6, CH_2), 68.29 (C-4, CH), 69.72 (C-5, CH), 71.35 (C-2, CH), 72.02 (C-3, CH), 91.45 (C-1, CH), 172.06 (C-1′, C=O).

Example 2: preparation of **B1** with continuous removal of methanol

In a 2-necked flask surmounted by a Soxhlet extractor (filled with activated molecular sieve), 0.5 mg of immobilized *Candida antarctica* B lipase (SP 435, manufacturer Novo Nordisk) was added to 0.9 g of D-glucose and 1.35 of palmitic acid methyl ester, followed by heating (ca. 60°C) with stirring (magnetic stirrer, 200 r.p.m.) under reflux and reduced pressure. The progress of the reaction was followed by thin-layer chromatography. After the end of the reaction, the reaction mixture was worked up as described in Example 1. **B1** was obtained in a yield of 67%.

Example 3: preparation of vitamin C esters

Vitamin C (ascorbic acid) was reacted with various carboxylic acid vinyl esters in the same way as described in Example 2 except that acetone/methanol (3:1) was used for extraction. The vitamin C esters shown in the following Table were obtained. Compounds **B2** and **B4** were additionally purified by extraction with chloroform/water (1:1). All the compounds thus obtained were characterized by NMR spectroscopy; the spectrum of **B4** is shown by way of example.

Compound	Reaction temperature	Reaction time	Yield
Ascorbyl palmitate (**B2**)	40°C	46 h	79%
Ascorbyl laurate (**B3**)	40°C	34 h	70%
Ascorbyl caproate (**B4**)	40°C	18 h	60 %

NMR spectrum of **B4**:

^{13}C-NMR (CD_3OD): δ (ppm) = 172.61 (COO in the ring of the ascorbyl group), 170.29 (C-1), 152.39 (COH in the ring of the ascorbyl group), 117.97 (COOH with COO in the ring of the ascorbyl group), 74.92 (CH in the ring of the ascorbyl group), 65.42 (CHOH ascorbyl group), 33.26 (C-2), 30.99 (C-6), 28.28 (C-4), 28.24 (C-5), 24.26 (C-3), 20.58 (C-7), 13.75 (C-8).

CLAIMS

1. A process for the production of polyols selectively esterified with carboxylic acids at the primary OH group, characterized in that the polyol is reacted with a carboxylic acid ester in the presence of an organic solvent and a hydrolase, preferably a lipase or esterase, as catalyst.

2. A process as claimed in claim 1, characterized in that the hydrolase is selected from the enzymes obtainable from Candida antarctica, Humicola lanuginosa, Rhizopus spec., Chromobacterium viscosum, Aspergillus niger, Candida rugosa, Penicillium camembertii, Rhizomucor miehei, Burkholderia spec. or Pseudomonas spec.

3. A process as claimed in claim 1 or 2, characterized in that the hydrolase is used in solid form, more particularly immobilized on a support material.

4. A process as claimed in any of claims 1 to 3, characterized in that the polyol is a sugar or sugar derivative.

5. A process as claimed in claim 4, characterized in that the sugar is selected from threose, erythrose, arabinose, lyxose, ribose, xylose, allose, altrose, galactose, glucose, gulose, idose, mannose, talose and fructose and di-, oligo- and optionally polymers composed of them.

6. A process as claimed in claim 4, characterized in that the sugar derivative is selected from the aldonic acids and ascorbic acid.

7. A process as claimed in any of claims 1 to 6, characterized in that the carboxylic acids correspond to the general formula R-COOH, where R is an optionally hydroxysubstituted alkyl or alkenyl group containing 6 to 32 carbon atoms or AR-$(CH_2)_n$ and AR is an optionally alkyl- or hydroxysubstituted phenyl or naphthyl group and n is a number of 0 to 4.

8. A process as claimed in any of claims 1 to 7, characterized in that the carboxylic acid is used in the form of lower alkyl esters, such as the methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert.butyl ester.

9. A process as claimed in any of claims 1 to 8, characterized in that the molar ratio between the carboxylic acid ester and the polyol deviates very little from 1 and, more particularly, is in the range from 0.8 to 1.2:.1

10. A process as claimed in any of claims 1 to 9, characterized in that organic solvent is used in 0.1 to 25 times and more particularly 0.5 to 18 times the quantity by weight of polyol to be esterified.

11. A process as claimed in any of claims 1 to 10, characterized in that the organic solvent is selected from dioxane, acetonitrile, acetone, γ-butyrolactone, tetrahydrofuran, tert.butanol, tert.amyl alcohol and 3-methyl-3-pentanol and mixtures thereof.

12. A process as claimed in any of claims 1 to 11, characterized in that it is carried out at temperatures in the range from room temperature to 80°C and more particularly at 60°C.

13. A process as claimed in any of claims 1 to 12, characterized in that an ester is used as the nonactivated carboxylic acid derivative and the alcohol released therefrom after reaction with the polyol is removed from the reaction mixture by azeotropic distillation.

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- [X] Attorney or agent of record.

Typed or Printed Name	John E. Drach, R.N. 32,891
Signature	John E Drach
Date	May 2, 2002

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing

Attorney Docket Number	C 2178 PCT/US
First Named Inventor	BORNSCHEUER, Uwe
COMPLETE IF KNOWN	
Application Number	10/009,316
Filing Date	05/02/2002
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:
My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR THE SELECTIVE ESTERIFICATION OF POLYOLES

(Title of the Invention)

the specification of which
☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 04/26/2000 as United States Application Number or PCT International

Application Number PCT/EP00/03764 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY	Priority Not Claimed	Certified Copy Attached? YES	NO
199 20 558.2	DE	05/05/1999	☐	☐	☒
199 24 221.6	DE	05/28/1999	☐	☐	☒

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U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
	PCT/EP00/03764	04/26/2000	

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

2-00

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